Oxidative Potential of Particulate Matter: Association with Metals, Polyaromatic Hydrocarbons and Meteorology

A

SYNOPSIS

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INTRODUCTION

The adverse health effects associated with atmospheric particulate matter (PM) have been well documented in epidemiological studies (Brunekreef and Holgate, 2002; Pope and Dockery, 2006; Russell and Brunekreef, 2009). It has recently been reported that $PM_{2.5}$ pollution contributed to nearly 3 million early deaths in 2017 and more than half of this disease burden fell on people living in China and India (SOGA 2019). A series of literature is linking exposure to PM with health outcomes, ranging from cardiovascular and respiratory diseases to neurological disorders (Li et al., 2008; Brook et al., 2010; Delfino et al., 2010; Morgan et al., 2011; Amatullaha et al., 2012; Rich et al., 2013; Beelen et al., 2014; Wang et al., 2015; Yu, et al.,2019). The generation of Reactive Oxygen Species (ROS) has been postulated to be an important mechanism leading to PM-induced toxicity and associated adverse health effects (Dellinger et al., 2001; Xia, 2007; Li et al., 2008; Saffari et al., 2014). ROS concentrations in excess of the antioxidant capacity of the body to neutralize them leads to oxidizing other cellular components, which eventually translates into numerous health outcomes (Peters et al., 2006; Li et al., 2009).

In the 1950s, Gerschman proposed that oxygen-containing free radicals were responsible for toxic effects in aerobic organisms (Gerschman et al., 1954). Over the years, the terms ROS (reactive oxygen species), ROI (reactive oxygen intermediates) and RNS (reactive nitrogen species) have been coined to define an emerging class of endogenous, highly reactive, oxygen- and also nitrogen- bearing molecules that includes families of oxygen-centered or related free radicals, ions, and molecules. This includes free radical hydroxyl ($OH^{\bullet}$), hydroperoxyl ($HOO^{\bullet}$), and organic peroxy radicals ($ROO^{\bullet}$). Ions includes the superoxide ($O_2^{-\bullet}$), hypochlorite (OCl^{-}), and peroxynitrite ($ONOO^{-}$) ions and molecules such as hydrogen peroxide (H_2O_2), organic and inorganic peroxides. Much of the attention has focused on the formation of ROS *in situ* after particle deposition in the respiratory tract generally through the interaction with transition metal ions (Stohs et al., 1995), organic hydrocarbons, such as polycyclic aromatic hydrocarbons and quinones (Squadrito et al., 2001) and ultrafine particle surfaces (Li et al., 2003).

Major sources of the atmospheric ROS include vehicular and industrial emissions, power plants and refuse incineration. Atmospheric ROS can be found in gas or particle-phase in the atmosphere. Particle-phase ROS are believed to be more harmful to human health than gas-phase ROS. This is because, gas-phase ROS have high water-solubility and molecular diffusivity, thus are more likely to be absorbed by mucus coating of the upper respiratory tract whereas particle-phase ROS can be deposited in lungs (Venkatachari et al., 2007; Chen et al., 2011). Organic compounds can generate oxidative stress through redox cycling of quinone-based radicals and metals can directly support electron transport to generate ROS while diminishing antioxidant levels (Ghio et al., 2012). Several researchers have investigated the redox activities of PM and their related health effects. These studies suggest that reactive oxygen species (ROS) are either produced by transportation into the lungs on respirable particles (exogenous ROS) (Zhao and Hopke, 2012) or inside the human body through a wide range of biochemical and physiological processes, by the inhaled PM containing transition metals such as Fe, Cu, Zn, Cr, Co, Ni etc., and/or organic compounds such as quinones, PAHs, and/or other unknown species in vivo (endogenous ROS) as shown in Fig-1. Endogenous ROS are involved in cell signalling processes and are naturally generated during cellular respiration reactions. Excessive amounts of ROS can have deleterious effects on the cell, including DNA damage and lipid peroxidation. To overcome this toxicity, organisms have evolved cellular defences called antioxidants, which help to neutralize ROS in the effort to maintain a healthy redox balance in the cell. The antioxidant defence system of cells consists of antioxidant enzymes such as superoxide dismutase, heme oxygenase-1 (HO-1), and antioxidant molecules like glutathione. A state of oxidative stress is reached when the production of ROS overpowers the antioxidant capabilities of the cell. Oxidative stress can alter the physiological functions of DNA, proteins, and lipids. In recent years, multiple studies have proposed the synergistic effects of oxidative stress and tissue inflammation to be responsible for age-related disorders, cancer, cardiovascular, inflammatory, and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Droge, 2002; Finkel et al., 2007; Dickinson et al., 2011). In general, the generation of ROS and the activity of antioxidant and radical scavenger defences are more or less balanced in vivo in healthy organisms.

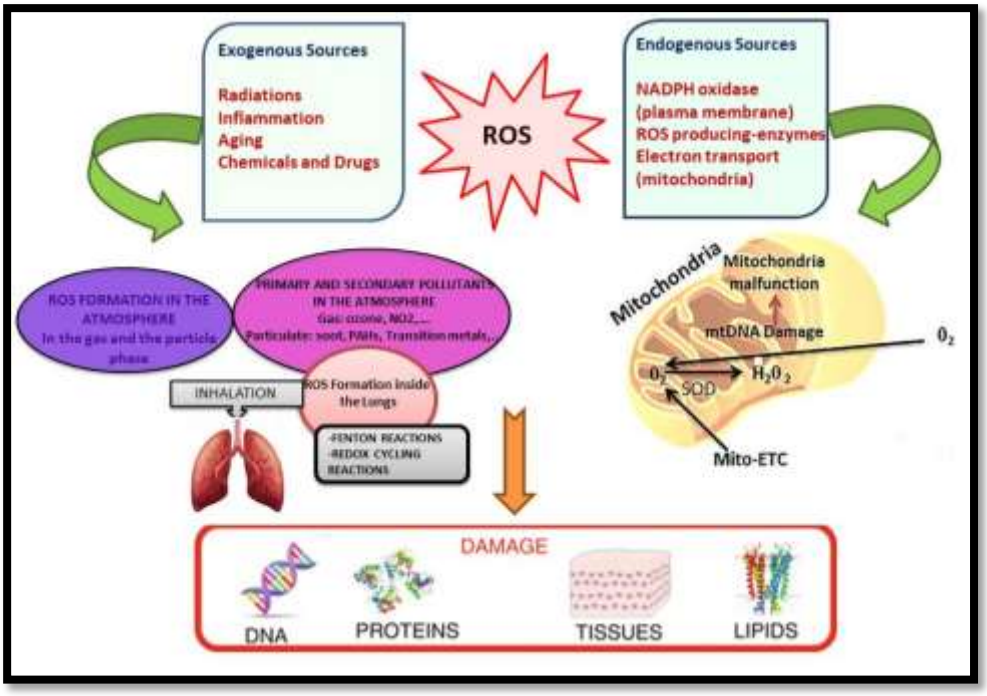


Fig-1. Exogenous and endogenous sources of Reactive Oxygen Species (ROS) and its damage

Several studies have investigated the relation of redox potential with chemical constituents of PM and found positive associations between redox potential measured by ROS activity and transition metals such as Fe, V, Ni, Cr, Cd, Zn and Pb (Hu et al., 2008; Verma et al., 2009, 2010). The presence of transition metals in PM can induce pro-inflammatory responses in human airways through their ability to form reactive oxygen species via Fenton or Fenton–like reactions in which hydrogen peroxide reacts with iron(II) in acidic medium (pH= 2.8-3.0) to form iron(III), hydroxyl anion and the highly reactive hydroxyl radical at room temperature (Vasquez-Medrano et al., 2018), as shown in reactions (1) and (2) (Prophete et al., 2006; Verma et al., 2010). In particular, water-soluble metals are the key drivers of oxidants generation (Knaapen et al., 2002; Cheung et al., 2012).

$$Fe^{2+} + H_2O_2 \xrightarrow[\text{room temp.}]{\text{pH= 2.8-3.0}} Fe^{3+} + {}^{\bullet}OH + OH^- \quad \text{.....(1)}$$

$$Fe^{3+} + H_2O_2 \xrightarrow[\text{room temp.}]{\text{pH=2.8-3.0}} Fe^{2+} + {}^{\bullet}OOH + H^+ \quad \text{.....(2)}$$

Fenton's Reaction

Although ROS is one of the most important constituent of atmospheric particulate matter with regard to its oxidative potential, its role in atmospheric chemistry and aerosol health effects, there are only a few measurements of ROS in the gas phase (e.g. OH, H_2O_2), and their distribution and concentration is relatively well-understood, whereas the measurements of ROS in aerosol particles is limited. Thus, assays have been developed to quantify the oxidative potential (OP) of PM. The recent development of acellular assays has led to a rapid rise in the measurements of redox activity of PM worldwide using varying methods with differing results. Acellular assays are currently faster and less resource intensive than cellular assays, allowing more rapid development of reasonably large data sets in different locations for use in source apportionment and health analyses. Electron spin resonance (ESR) with 5,5-dimethylpyrroline-N-oxide (DMPO) as a spin trap, measures the ability of PM to induce hydroxyl radicals ($^{\bullet}OH$) in the presence of H_2O_2 catalysed by transition metal (Boogaard et al., 2012; Shi et al., 2003). Ascorbic acid (AA) is a vital water-soluble antioxidant found in living organisms. The anion of ascorbic acid, ascorbate acts as a reducing agent, donating electrons to various metabolic reactions in biological system. Its reducing power makes it a powerful antioxidant, rapidly scavenging many reactive oxygen species (ROS). The Ascorbic acid assays would be more specific to the oxidative potential of transition metals (Godri et al., 2011; Yang et al., 2014), but ascorbic acid is known to react with organics such as quinones (Shang et al., 2012; Visentin et al., 2016). The Dithiothreitol (DTT) assay is used to measure the oxidative potential of particles and has frequently been applied to atmospheric PM and major PM sources. It is a purely chemical method, in the assay redox-active chemicals in the PM oxidize added DTT to its disulfide form and the linear rate of DTT loss is equated to the oxidative activity of the PM. In the process an electron transfer from these redox-active species to molecular oxygen occurs, forming superoxide which can react to form other ROS species (e.g. hydrogen peroxide and hydroxyl radical) (Cho et al., 2005, Charrier and Anastasio, 2012; Yu et al., 2018). The technique is responsive to redox-active organic compounds (e.g. quinones) and many metals (Cho et al., 2005; Charrier and Anastasio, 2012; Lin and Yu, 2011). Due to high detection sensitivity, fluorescent-based probes have also been used to quantify PM-related ROS. These are based on the principle that a fluorescent product is generated when the non-fluorescent probe molecule reacts with ROS. The most common used probe is 2,7-dichlorofluorescein

(DCFH) (Fuller at al., 2014; Wang et al., 2011; Venkatachari et al., 2008). Other common assays involve measuring the ability of PM to deplete antioxidants such as vitamin C, glutathione and uric acid (Mudway et al., 2004). All assays are responsive to metals, though the types of metals that drive responses and the degrees of those responses vary by assay. For example, AA is more sensitive to iron and copper and DTT to copper and manganese (Bates et al., 2019).

PM oxidative burden is an appealing exposure metric for epidemiological analysis as it aims to capture the ability of PM to modify a biological process to contribute in the adverse cardiorespiratory health effects. The growing literature on the relationship between oxidative potential and health, specifically cardiorespiratory endpoints need to evaluate the effect of the chemical speciation of particulate matter and emission sources with redox activity of PM to better understand the link between PM and health. So, it is important to understand how the ROS concentration on respirable PM varies as environmental conditions change. Thus, the main focus of this study is to determine the concentration of ROS in size-fractionated (PM_{10}, $PM_{2.5}$, and PM_1) ambient particulate matter, their association with metals, polyaromatic hydrocarbons, and meteorology.

LITERATURE REVIEW

INTERNATIONAL STATUS

In recent years, several studies on ROS have been conducted in various cities of the world. These studies identified and quantified ROS in samples of $PM_{2.5}$ collected from different environments (Verma et al., 2014; Khurshid et al., 2014; Crobeddu et al., 2017). The ROS-generation potential has been evaluated in several urban sites like parking garage in University of Illinois Urbana−Champaign campus (North Campus Parking), Illinois, United States (Xiong et al.; 2017), Jefferson Street and Georgia in Atlanta, USA (Bates et al., 2015; King and Weber, 2013), Bern (Switzerland), Beijing (China) (Zhou et al., 2015) and Austin (Texas) (Khurshid et al., 2014) in $PM_{2.5}$. The diurnal variations of reactive oxygen species (ROS) in various size fractions of ambient particles were determined by Venkatachari et al. in Rubidoux, CA. They observed that smaller particles, especially particles in the 10–56 nm range, were observed to have higher ROS concentrations and the ROS production at night was found to be comparable to daytime production. They also confirmed that the intensity of photochemical reactions was only a moderate factor affecting the daytime ROS concentration (Venkatachari et al., 2005).

The ROS activity of fog droplets is even greater (> 2.5 times) than that of the PM on which droplets are formed, indicating that redox-active compounds are not only scavenged from the particulate phase, but are also produced within the droplets. Fog formation exerts a scavenging effect on PM mass and redox active compounds, the aqueous-phase formation of reactive secondary organic compounds can eventually enhance ROS activity of PM when fog evaporates. Airborne toxicity in the Po Valley is largely produced during fog formation and fog processing and not by the emission/transport of pollutants. With very limited traffic emissions at the Po Valley, the ROS levels recorded for samples during fog period were 4142 ± 1347 μg zymosan mg and simultaneously for PM samples were 1879 ± 851 and 1873 ± 1199 μg zymosan mg during day and night respectively (Decesari et al., 2017). Gali et al. studied the diurnal variation and oxidative potential of PM and semi-volatile-PM at roadside and a background site in Hong Kong during both summer and winter seasons.

The diurnal comparisons showed 10-20% higher cellular oxidative stress during afternoon period at the roadside than at night time at the background site (Gali et al., 2019).

Hung and Wang 2001 determined the concentrations of reactive oxygen species (ROS) in various particle size fractions for the Taipei aerosols and for particles in vehicular exhausts. They observed that the concentration of ROS in ambient particles had good correlations with the intensity of photochemical reactions which were a major factor affecting ROS concentration in smaller particles, especially in ultrafine particles. The correlation coefficient between the ROS concentration and the O_3 concentration was higher for smaller particles (Hung and Wang 2001). Ntziachristos et al. 2007 showed that the organic component of the PM samples is an important factor in determining redox activity, and that both carbon and organic compounds are important in the redox activity of PM, even within particle size modes (Ntziachristos et al., 2007). Good correlation between the redox activity and several PM species, including organic carbon, trace metals, and functionalized aromatic, and polar organic compounds has been observed in urban area as compared to rural area (Geller et al. 2004, Jeng et al., 2010). DCFH assay is used to detect ROS in the particle phase of PM_1 in different urban sites of Bern and Beijing. ROS concentrations in Bern were between 0.2-5 nmol/m^3 and in Beijing near to 13 nmol/m^3. However, the ROS content per particle mass was on average 0.26 nmol/µg in Bern and 0.15 nmol/µg in Beijing (only moderate and haze days) (Zhou et al., 2015). The rate of generation of ROS catalyzed by ambient $PM_{2.5}$ collected from urban site in the DTT assay and suggested that Cu(II) was dominant metal in DTT oxidation, contributes almost negligibly to the ROS generation whereas Fe is mostly inactive in DTT oxidation, but shows synergistic effect in •OH formation in the presence of other quinones (Xiong et al.; 2017).

The redox activity of diesel exhaust particles (DEP) can vary based on engine configuration, driving conditions, photochemical aging of exhaust and fuel type. Gasoline and diesel vehicles are a major source of urban PM (Schauer et al., 2002; Fraser et al., 2003), and the oxidative stress of Diesel Exhaust Particles (DEP) exposure are of interest in many studies (Li et al., 2002, 2003; Zhou et al., 2018). Chemical analyses of diesel exhaust particles revealed that the aromatic fractions are enriched

with polycyclic aromatic hydrocarbons (PAHs), and polar fractions are enriched with redox-cycling quinones which can persist for months and reduce oxygen to produce superoxide anion, hydrogen peroxide, and hydroxyl radicals via redox cycling (Penning et al. 1999; Dellinger et al. 2001).

ROS potential of different types of aged atmospheric particles in the presence and absence of an urban hydrocarbon environment was assessed by Rattanavaraha et al. This study concluded that diesel exhaust PM had a higher ROS response when it was in the presence of an urban hydrocarbon mixture and was associated with significant O_3 production. For all the aged dilute diesel systems, ROS expression increased by a factor of 2-4 over fresh diesel particles (Rattanavaraha et. al., 2011). Literature shows that PM related to vehicle emissions drives responses in all assays, most likely due to the high copper content in brake and tire wear, and biomass burning PM significantly contributes to DTT (dithiothreitol) assay due to its large oxidized aromatic (e.g., quinone) fraction (Bates et al., 2019) . Water soluble $PM_{2.5}$ at Atlanta, in the period of 1998-2009 was examined by Bates et al. Linear regression modeling was used to relate $PM_{2.5}$ emissions sources to ROS-generation potential and to estimate DTT activity. They observed that light-duty gasoline vehicles exhibited the highest intrinsic DTT activity, followed by and heavy-duty diesel vehicles (0.11 ± 0.02, 0.069 ± 0.02, and 0.052 ± 0.01 nmol min^{-1} μg^{-1}source, respectively). Biomass burning contributed the largest fraction to total DTT activity over the study period, followed by light-duty gasoline vehicles and heavy-duty diesel vehicles (45%, 20% and 14%, respectively) (Bates et al., 2015).

The dithiothreitol (DTT)-based chemical reactivity is considered as quantitative method for the assessment of the capacity of a PM sample to catalyze ROS generation. The major components of the ambient PM identified so far to be associated with the DTT activity are often bulk groups of species such as organic carbon (OC), both water-soluble (WSOC) and insoluble (WIOC) and water-soluble HULIS (humic-like-substance) compounds (a class of WSOC characterized by strong hydrophobicity). However, most of these associations have been inferred based on statistical correlations (Ntziachristos et al., 2007; Hu et al., 2008; Biswas et al., 2009; Verma et al., 2009). Kumagai et al. monitored the reaction in presence of excess and observed the redox-active quinone

9,10-phenanthraquinone can effectively catalyse the transfer of electrons from DTT to oxygen, generating superoxide. Li et al. showed that the consumption rate of DTT by PM samples is directly related to the particles' ability to induce stress protein formation in cells and DTT linearly correlated with heme oxygenase-1 induction and glutathione depletion. Cho et al. demonstrated that the DTT assay can provide a good measure of the redox activity of particles by determining superoxide radical formation (Cho et al. 2005). The diurnal variations in the concentrations of ROS in various size fractions observed for ambient particles in Rubidoux, CA, that suggested the correlations between ROS and ozone, particularly in the early to mid-afternoon (Venkatachari et al., 2005). Ntziachristos et al. 2007 showed a significant correlation between DTT and organic carbon (OC) levels in PM samples from various sites throughout the Los Angeles Basin. In a recent study, certain transition metals (e.g., Fe, Cu, and Mn) have also been shown to be active in this assay (Charrier and Anastasio, 2012). Verma et al. assessed the relative contributions of water and methanol-soluble compounds and their hydrophobic/hydrophilic subfractions to the ROS-generating potential of ambient fine aerosols at an urban site near central Atlanta during January- February 2012 by the dithiothreitol (DTT) assay. Their findings suggest that the hydrophobic components of both water-soluble and insoluble organic aerosols substantially contribute to the oxidative properties of ambient PM (Verma et al., 2012). Verma et al. showed that the DTT activity of ambient fine aerosols collected from various sites and seasons in the southeastern US is generally correlated with PM mass; however, the strength and slope of the correlation varied (r^2 = 0:40-0:90; slope=0.01-0.05 nmol min^{-1} μg^{-1}) among different sites and seasons (Verma et al., 2014).

Currently, the DCFH assay is one of the most commonly used assays. Accurate ROS quantification remains challenging because some ROS are highly reactive and are likely at least partially degraded before measurement when using offline techniques, which typically have delays of hours, days or weeks. DCFH assay has a good sensitivity for ROS measurement in both cellular and acellular application (Hung and Wang, 2001; Venkatachari et al., 2007; Zhang et al., 2008). Khurshid (2014) assessed the seasonal variations in particulate ROS concentrations in samples of $PM_{2.5}$ in Austin, Texas. Particulate ROS concentrations measured were within the range of 0.0–5.7 n moles $m^{-3,}$

reported in several studies performed in United States (Hewitt and Kok, 1991), Taiwan (Hung and Wang, 2001), and New York, United States of America (Venkatachari et al., 2005, 2007).

ROS are usually quantified with off-line techniques in extracts of aerosol particles collected on filters (Hung and Wang, 2001; Venkatachari et al., 2005). Due to the reactive nature of ROS, such off-line methods risk severely underestimating the true ROS concentration in particles because some ROS components (e.g., radicals or peroxides) might decompose during sample collection and work-up. Therefore, two recent studies presented on-line and semi on-line techniques to quantify particle-bound ROS with DCFH to reduce the time between collection and analysis associated with filter collection and for higher time resolution measurements (Wang et al., 2011; King and Weber, 2013). King and Weber (2013) developed an online, semi-continuous instrument to measure gas and particle-phase reactive oxygen species (ROS) based on the fluorescent probe 2′7′- dichlorofluorescein (DCFH) at urban and rural sites in Atlanta. Zhou et al. (2015) used the DCFH assay and the on-line reactive oxygen species (ROS) analyzer to detect and compare ROS in the particle phase of ambient aerosol in different urban areas of Bern (Switzerland) and Beijing (China). A semi-automated system to quantify oxidative potential of filter aqueous extracts utilizing the dithiothreitol (DTT) assay a similar semi-automated system for the ascorbic acid (AA) assay was developed by Fang et al., (2016) and used to analyze AA and DTT activities and various aerosol species in $PM_{2.5}$ filter samples collected in different locations in the southeastern US. The developed on-line ROS analyzer could be applied to ambient air measurement. However, none of these on-line instruments is commercially available, which limits their widespread use. Therefore, online techniques (through direct sampling into the liquid phase and measurement within a few minutes) are necessary for reliable ROS quantification (Wragg et al., 2016).

The formation of ROS was reported in aqueous mixtures of secondary organic aerosols (SOA) and mineral dust by applying electron paramagnetic resonance (EPR) spectrometry. ROS formation can be explained by the decomposition of organic hydroperoxides, which are a prominent fraction of SOA, through interactions with water and Fenton-like reactions with dissolved transition metal ions which implies the

chemical reactivity and aging of SOA particles can be enhanced upon interaction with mineral dust in particles or cloud/fog droplets. SOA decomposition could be comparably important to the classical Fenton reaction of H_2O_2 with Fe^{2+} and that SOA can be the main source of OH radicals in aqueous droplets at low concentrations of H_2O_2 and Fe^{2+} (Tong et al., 2017). Real time chemical characterization of non-refractory submicron aerosols (NR-PM_1) during post monsoon (September/October) at a polluted urban location of Kanpur, India was reported by Chakraborty et al. (2017). Organic aerosol (OA) was found to be the dominant species with 58% contribution to total NR-PM_1 mass, followed by sulfate (16%). The study revealed the dominance of secondary/oxidized OAs in all seasons which shows the maximum oxygenation capacity of the environment to produce secondary OA from the primary emissions (Chakraborty et al., 2017). Zhang et al., 2008 investigated the associations between atmospheric particulate matter sources and in vitro ROS activities by measuring the ambient concentrations of fine particle water-soluble elements and total organic and elemental carbon in Denver in 2003 and applied multivariate factor analysis source apportionment model, positive matrix factorization (PMF) and determined the contributions of nine sources or factors: a mobile source factor, a water-soluble carbon factor, a sulphate factor, a soil dust source, an iron source, two-point sources characterized by water-soluble toxic metals, a pyrotechnic factor, and a platinum group metal factor. Results show that PM-stimulated in vitro ROS production was significantly positively correlated with the contributions from three sources: the iron source, the soil dust source, and the water-soluble carbon factor. The iron source accounted for the greatest fraction of the measured variability in redox activity, followed by the soil dust and the water-soluble carbon factor. Seventy-seven percent of the in vitro ROS activity was explained by a linear combination of these three source contributions (Zhang et al., 2008).

Endogenous ROS plays an important role in the maintenance and alteration of cellular processes governing hormone concentration, chemical equilibrium and enzyme activation (Powers and Jackson, 2008). The effects of ROS are evident in the skeletal muscle, the immune system and the nervous system. In irradiated cells, levels of these reactive species may be increased due to perturbations in oxidative metabolism and chronic inflammatory responses, thereby contributing to

long-term effects of exposure to ionizing radiation on genomic stability (Azzam et al., 2012). Hydroxyl radical generated by irradiation may induce oxidative damage of the mitochondria through peroxidation of the phospholipids and then enhance the generation of O^{2-} by the mitochondria. After gamma-ray irradiation, the intracellular ROS levels were detected by DCFH assay. Fluorescence increased robustly within minutes and then disappeared within 30 min because ionizing radiation can ionize H_2O into $^{\bullet}OH$, a toxic form of ROS with a life span as short as 10^{-9} s (Yoshida et al., 2012).

NATIONAL STATUS

Poor air quality is a growing societal concern in India. High concentration of PM has been documented over different regions of India that may be characterized by extremely high levels of redox activity of PM. Studies on oxidative potential of ambient aerosols, are limited in India (Rastogi and Patel, 2017; Patel and Rastogi, 2018b).

Sequential extraction of particulate matter (PM)-bound metals and the potential health risks associated with them in a growing metropolitan city (Pune) of India was examined by Roy et al., 2016. Average mass concentration of both PM_{10} and $PM_{2.5}$ exceeded the National Ambient Air Quality Standards. Significant seasonal variation in mass concentration was found for both size fractions of PM with higher values in winter season and lower in monsoon. Combustion of fossil fuels, coal, re-suspension of household dust, waste dumping, paint, candle burning, and outdoor impact identified as the possible common sources by Factor analysis. The presence of free radicals in PM samples collected from rural and urban site of Pune was confirmed by EPR. In both PM_{10} and $PM_{2.5}$, EPR spectra were attributed to both Fe^{3+} (g = 1.99~2.0) and free radicals (g = 2.0023 and g = 2.003) in PM. The study suggested that Fe plays an important role in the oxidant generating activity of PM. Dithiothreitol (DTT) assay was also performed which supports the presence of higher redox activity in the fine fraction of PM at the rural site (Roy et.al, 2016).

The possible genotoxicity of ambient particulate matter (PM_{10} and $PM_{2.5}$) in Pune city was observed by Yadav et al., 2018. In both size fractions of PM, Fe was found to be the dominant metal by concentration, contributing 22% and 30% to the total mass of metals in PM_{10} and $PM_{2.5}$, respectively. The speciation of soluble Fe in PM_{10} and $PM_{2.5}$ was investigated. The average fraction of Fe^{3+} and Fe^{2+} concentrations in $PM_{2.5}$ was 80.6% and 19.3%, respectively, while in $PM_{2.5}$ this fraction was 71.1% and 29.9%, respectively. The dominance of Fe (III) state in both PM fractions facilitates the generation of hydroxyl radicals, which can damage deoxyribose nucleic acid (DNA), as was

evident from the gel electrophoresis study. This study specified that air particle pollution may cause severe health disorders to the residents of this city (Yadav et.al. 2018).

Particulates from engine exhaust have potential to increase the ROS activity in cells which results in an elevated oxidative stress level. PM samples from the exhaust of different engines exhibited cytotoxic effect and showed the significant potential for generation of reactive oxygen species. The study on biodiesel-fuelled engine exhaust was observed by Agarwal et al. that revealed the presence of several harmful PAHs and trace metals which affected the biological activity of PM. It was also confirmed from this study that modern engines produced relatively cleaner exhaust compared to older engines (Agarwal et al. 2018a). In the investigation of the toxic potential, particulates emitted from diesel and gasoline engines showed higher mutagenicity, while those from CNG engine showed negligible mutagenicity. It was also observed that PAHs adsorbed onto particulates emitted from CNG engines were also relatively fewer compared to those from diesel and gasoline engines (Agarwal et al. 2018b).

The toxicological profile of 13 polycyclic aromatic hydrocarbons (PAHs) associated with airborne PM was assessed by Roy et al., 2019. The average concentration of both PM_{10} and $PM_{2.5}$ exceeded the Indian National Ambient Air Quality Standards. The combined results derived from diagnostic ratios and factor analysis suggested that the vehicular emission (Diesel + gasoline) and coal combustion were probable sources of PM bound PAHs. The redox activity measured by DTT assay exhibited the oxidative nature of PAHs associated with both sized PM (Roy et al., 2019).

A number of studies are reported from the Indo-Gangetic Plain (IGP), India which receives large emissions of aerosol and their precursors from various anthropogenic sources such as large-scale post-harvest biomass burning, vehicles, industries, fossil fuel burning and bio-fuel burning (Rajput et al., 2014; Singh et al., 2016; Singh et al., 2017; Satish et al., 2017). Despite the dominance of pollutants over the IGP, oxidative potential of ambient PM over this region is less studied (Rastogi et al., 2017). The study of the oxidative potential of fine aerosol ($PM_{2.5}$) at Patiala, a semi-urban site in

the IGP during the foggy and non-foggy nights has recently been conducted by Patel et al. (2018). It was observed that carbonaceous aerosol derived from biomass burning was more DTT active as compared to those derived from fossil fuel burning due to the production of redox-active species by fog processing (Patel et al., 2018a). Seasonally averaged mass-normalized oxidative potential values were 14±8, 14±7, 10±7, and 23±9 pmol DTT min^{-1} μg^{-1} during winter, spring, pre-monsoon and post-monsoon, respectively in a study carried out at Mount Abu, India, from March 2014 to May 2016. The highest DTTm values found during post-monsoon were attributed to long-range transport of anthropogenically emitted pollutants brought by the northeasterly (NE) air-masses (Patel and Rastogi, 2018b).

Several studies related to heavy metals in TSP, $PM_{2.5}$, and PM_{10} along with determination of their metal fractionation, size distribution, seasonal and spatial variations and identification of their sources are reported from Agra (Lakhani et al., 2008; Kulshrestha et al., 2009; Kulshrestha et al., 2014; Sah.et al., 2018). The study for the bioavailability and human health risks of As, Pb, Ni, Co, Cr and Cd in fine particulate matter ($PM_{2.5}$) at an urban site were investigated and observed that Cd and Co had high bioavailability. Ingestion is the major exposure pathway for all heavy metals except Cr to infants, children and adults followed by inhalation and dermal contact (Sah.et al., 2017). Sah et al., 2019 reported higher concentration of Fe in PM_{10} and observed that the carcinogenic risks of As, Cd, Co, Cr, Pb and Ni for both children and adults via dermal contact and ingestion exposure were within the acceptable level ($<1 \times 10^{-4}$) (Sah et al., 2018). Another study were carried out for the distribution and chemical fractionation of heavy metals in $PM_{2.5}$ collected rural site in Agra which showed that Pb and Ni are readily mobilized and more bioavailable (Sah et al., 2019).

Fewer have also evaluated the mutagenic potential and associated health risk through monitoring of PAHs levels in both $PM_{2.5}$ and PM_{10} collected from different site in Agra (Singla et al., 2012; Dubey et al., 2015). Recently, Verma et al., 2017 investigated the gas–particle partitioning of polycyclic aromatic hydrocarbons (PAHs) in $PM_{2.5}$ collected from a rural and a traffic site. The total

concentration of PAHs (gas + particulate) was 4015 and 624 ng m^{-3} at the traffic and rural sites respectively (Verma et al., 2017).

The plausible biological mechanisms explaining $PM_{2.5}$ health effects, oxidative stress is often cited as playing an important role in both respiratory and cardiovascular outcomes. For this reason, recent attention has focused on PM oxidative burden as an approach to characterizing particle toxicity. However, none of the studies have so far linked the chemical components and sources of ambient PM to its oxidative potential with concerning health impacts in this city. The focus of the study is to measure the oxidative potential of ambient particulate matter through in vivo (acellular methods) ROS assays and examine the role of organic species (PAHs) and metals to generate oxidation potential. The influence of meteorological conditions on the particulate ROS with the spatial and temporal characteristics of different size of PM will also be observed.

OBJECTIVES

Air pollution is a major threat to health and a matter of serious concern. Studies from several urban sites indicate that the $PM_{2.5}$ levels exceed the prescribed limits of NAAQS (India) WHO and USEPA. Agra is a major urban population centre with relatively high emissions of particulate matter from a variety of sources including transport, mobile sources, etc. and from secondary aerosol formation. Despite their environmental relevance, no study has been conducted on ROS generation in atmosphere at Agra in the particulate phase. Hence this study has been planned to:

- Assess the potential of the water-soluble fraction of atmospheric fine aerosols to generate reactive oxygen species (ROS).
- Investigate the spatial and temporal characteristics of PM redox activity.
- Assess the influence of meteorological conditions on these particulate ROS concentrations.
- Assess the role of organic species (PAHs) and metals, (particularly the bioavailable fraction of metals) in generating redox activity.
- Identify major ROS-associated emission sources.
- Case Study – Influence of vehicular traffic density on PM levels during weekdays and weekends.

PROPOSED METHODOLOGY

Sampling Sites

Agra is a major urban population centre with relatively high emissions of particulate matter from a variety of sources including mobile sources, biomass burning, etc. and secondary aerosol formation, making it a suitable location to study particle toxicity. Sampling will be conducted at two sites simultaneously, Bhagwan talkies, a site influenced by anthropogenic activity and D.E.I. campus with minimum anthropogenic activity.

Sample Collection and Measurement

Sample Collection

Ambient particulate samples (PM_{10}, $_{2.5}$ and $_{1}$) will be collected once in a week. Twenty-four hour integrated sampling of PM_{10} and $PM_{2.5}$ will be carried out with fine particulate dust sampler (APM 550, Envirotech) on pre-weighed and pre-conditioned quartz-fibre filters at a flow rate of 16.6 litres per minute. For PM_1 samples, particulate Sampler Envirotech APM 577 will be operated at a constant flow rate of 10 L/min. For measurement of mass concentrations, the filters will be weighed before and after each sampling using a Mettler Microbalance. All filter samples will be wrapped in prebaked aluminium foil and stored in a freezer (−20 °C) till the analysis completed. Meteorological parameters like temperature, solar radiation, wind speed, wind direction, and relative humidity will be recorded by Envirotech WM 271 Monitor.

ROS Analysis

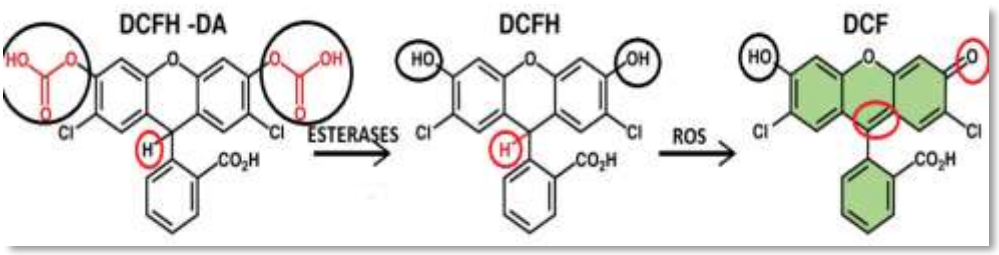
After sampling, one-fourth of the filter paper will be extracted into 10ml ultrapure water (specific resistance, 18Ωcm) by using the ultrasonic baths for 45 min. The extracted solution will be filtered and analysed by the DTT and DCFH assay for ROS generation potential.

DTT Assay

0.1 mL of the PM sample with 5mL of DTT (100 µM) in 10 mL of 0.1M potassium phosphate buffer at pH 7.4 will be incubated at 37˚C for times varying from 15 to 90 min. At the designated times, 0.5 mL of 10% trichloroacetic acid (TCA) will be added to the incubation mixture and a 0.5-mL aliquot of the reaction mixture mixed with 2.0 mL of 0.4M Tris-HCl, pH 8.9 containing 20mM EDTA and 50 µL of 10mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB). The concentration of the formed 5-mercapto-2- nitrobenzoic acid will be measured by its optical density absorption at 412 nm by UV-spectrophotometer. An operative blank will be always measured in parallel (Charrier and Anastasio, 2012).

The DCFH Assay

The DCFH procedure will be adapted from Huang et al., 2016. Operating in the dark, 5.0 mg of 2′,7′-Dichlorofluorescin diacetate will be dissolved in 5.0 mL of ethanol and mixed with 20.0 mL of 0.01M NaOH in order to favour de-acetalisation. The 5.0 µM DCFH solution will be kept then in the dark at room temperature for 30 min before use. The HRP solution (0.5 units mL^{-1}) will be prepared by dissolving in phosphate buffer 25.0 mM at pH 7.4 (in 1 L). It will be at 37 °C for 15 min before use. Aliquots of 5.0 mL of the HRP solution will be mixed with 1.5 mL of the sample (soluble or residual fraction) and with 125 µL of the DCFH solution. The mixture will be incubated again at 37 °C for 15 min. After incubation, samples of particle suspension in 0.1 ml aliquots will be used to analyse fluorescence intensity of DCF by a Fluorescence Spectrometer at the emitted radiation 530 nm (excitation wavelength 427 nm). The OP values will be obtained using the calibration curve to convert the obtained fluorescence intensity into H_2O_2 equivalents.



Non- Fluorescent Non-Fluorescent Fluorescent

Analysis of PAH

One-fourth portion of the filter paper will be cut into small pieces. The extraction of these pieces will be performed in 50 ml mixture of DCM and n-hexane (4:1 ratio) using ultrasonication for 60 min. The sonicated extracts will be filtered through filter papers, cleaned by employing a silica gel column, will be concentrated up to 2 mL by rotavapor (BUCHI) at a bath temperature of ≤40°C and stored in aluminium foil wrapped glass vials at a low temperature until analysis.

PAHs will be analyzed in selected ion monitoring mode according to their m/z ratio by using a Gas Chromatograph-Mass Spectrometer (GC-MS, BRUKER SCIONSQ). Separation will be achieved on an Rtx-5 MS capillary column {30 m in length, 0.25 mm internal diameter and 0.25 μm df containing cross linked dimethyl poly siloxane, (Cat no. 12623)}. Helium will be used as the carrier gas at a flow rate of 1mL m^{-1}. The temperature of the oven will be set at 60 ^{0}C for 4 minutes, and it will be increased to 290 ^{0}C at a rate of 8 ^{0}C min^{-1} and will be held for 30 min. The temperature of the injector will be maintained at 270 ^{0}C. The mass scanning will be ranged between 50 and 500. For each analysis 1 mL sample will be injected into the GC-MS in split less mode for the entire duration of analysis (62 minutes).

Analysis of Metals

Due to potential toxicity of heavy metals to human life and environment, determining the chemical form of heavy metals in atmosphere is an important approach and can provide useful information on its mobility, bioavailability and the potential risks. Sequential extraction methods have been widely applied to characterize the chemical forms of metal element present in various environmental samples (Smichowski et al., 2005; Mukhtar and Limbeck 2013). For this analysis of metal, one half of the loaded quartz fibre filter will be sequentially extracted according to the procedure developed by Espinosa et al., (2002). This method fractionates the metals into water soluble, environmentally mobile (F1), bound to carbonate and oxides (F2), and environmentally immobile fraction bound to organic matter (F3) and silicates (F4). Elements in F1 and F2 fractions are considered as bioavailable fractions as they can interact with human body fluid when they enter the

body through inhalation and/or oral exposure. Therefore, the percentage of the first two fractions (F1 and F2) to the total metal concentration (F1 + F2 + F3 + F4) is termed as bioavailability index (BI), which is used to evaluate the bioavailable potential of a metal (Richter et al., 2007; Feng et al., 2009; Schleicher et al., 2011).

The reagents and operational conditions to be followed in each step are given below in table 1.

Table 1: Chemical fractions, reagents used and operational conditions of the sequential extraction procedure modified after Fernandez Espinosa et al., (2002).

Metallic fraction	Mobility	Reagent	Experimental conditions
F1. Soluble and exchangeable metals	Highly Mobile	30 mL H_2O (pH =7.4)	3 h shaker agitation at room temperature + reduce the volume to 1–2 mL by heating on hot plate + made to 30 mL with 2% HNO_3 + stored at 4 °C until analysis
F2. Carbonates, oxides and reducible metals	Mobile	30 mL $NH_2OH.HCl$ 0.25 M at pH 2.0	5 h shaker agitation at room temperature + reduce the volume to 1–2 mL by heating on hot plate + made to 30 mL with de-ionized water.
F3. Bound to organic matter, oxidizable and sulphidic metals	Less Mobile	15 mL NH_4OAc 2.5 M at pH = 3.0 + 15 mL H_2O_2 30% + 15 mL H_2O_2 30%	Shaker agitation at 95 ^oC until near dryness + repetition + Shaker agitation for 90 min at room temperature+ reduced to 1–2 mL on a hot plate + made up to 30 mL with de-ionized water.
F4. Residual metals	Immobile	10 mL (HNO_3:HCl:$HClO_4$) (6:2:5)	Shaker agitation for 5 h at 95 ^oC and evaporating to dryness + made up to 50 mL with de-ionized water.

Concentration of metals in each fraction will be determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES). Instrument will be calibrated by the standard ranging between 0.1 to 10.0 ppm and samples will be analyzed in the same way. Concentrations of all species will be corrected for corresponding blanks.

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